

Amendments to the Specification:

Please replace paragraphs 42, 43, 59, 61, 69, 71, 82, and 86 with the following amended paragraphs:

[0042] Lyophilized beads can, but need not, have all components required to complete an amplification reaction. For example, in some circumstances it is convenient to store a mixture of some, but not all, of the components required for an amplification reaction. In some cases, all components but the nucleic acids are in the lyophilized bead. In some embodiments, only the components that are stable at room temperature or in a lyophilized mixture are included. In some aspects, the lyophilized beads are used in a microfluidic device. Exemplary microfluidic devices that employ amplification reaction mixtures include, *e.g.*, the SmartCycler SMARTCYCLER®, GeneXpert GENEXPERT® and I-CORE® devices (Cepheid, Sunnyvale, CA). However, the advantages of the invention extend to all microfluidic devices, whether for use in amplification reactions or not.

[0043] Thus, in some aspects, the amplification mixture comprises a buffer, excipient, a carrier protein, magnesium, and an antifoam agent. Lyophilized beads that contain these mixtures are known as "Blank Beads". In some aspects, the reaction mixture also comprises a polymerase such as a DNA polymerase (*Taq* polymerase, polymerases complexed with hot start antibodies such as Platinum Platinum® polymerases (Invitrogen, Carlsbad, CA)), RNA polymerase, reverse transcriptase, and/or deoxynucleoside triphosphates (*e.g.*, dATP, dCTP, dTTP, dGTP). Lyophilized beads that contain these mixtures are known as "Generic Beads". In some embodiments, the amplification mixtures will comprise primers which correspond a particular DNA sequence of interest, as well as probes which will detect the presence of primer hybridization with the DNA sequence of interest. Lyophilized beads that contain these mixtures are known as "Assay Specific Beads". Examples of bead buffer formulations of the invention are found in Example 1.

[0059] The composition and shape of the drying surface for the dispensed emulsion is important as it determines the drop shape and the ease of release from the surface after drying. In some embodiments, the dispensed emulsion is placed upon glass, polystyrene, wax paper, ~~Delrin~~ DELRIN[®] or a coated aluminum pan (coatings can be nickel, ~~Teflon~~ TEFLON[®], titanium nitride and combinations thereof).

[0061] Vacuum drying, evaporation, and freeze-drying of the solution can be utilized for drying the bead material. A standard freeze-drier (such as a ~~VirTis~~ VIRTIS[®] Advantage and/or GENESIS) with a control modified to allow operation at partial vacuums can be used. Additionally, a constant purge of dry nitrogen into the chamber can provide gas flow to carry moisture away from the bead material.

[0069] Bead formation was conducted using the IVEK ~~Digispense~~ DIGISPENSE[™] 700 system (IVEK Corporation, North Springfield, VT) dispensing system. The system was loaded with the liquid bead buffer formulation, which was dispensed in 12.50 μ L amounts into liquefied nitrogen contained in a Dewar flask with insert dividers. Dispensing the liquid bead buffer formulation into liquid nitrogen caused spontaneous bead formation. Bead freezing required submersion in the liquid nitrogen for approximately 20 to 30 seconds. During this period, the beads were maintained in separate areas by the insert dividers to prevent bead fusion.

[0071] The lyophilization cycle parameters are shown in Table 2. A ~~VirTis~~ VIRTIS[®] Advantage 2.0 EL lyophilizer (VirTis, Gardiner, New York) was used.

[0082] Residual moisture determination was performed on a Mettler Toledo DL36 KF Coulometer attached to a GA42 Mettler Toledo printer and AG104 Mettler Toledo electronic balance. Moisture experiments were performed using five sets of 28 lyophilized blank beads containing 64.6% (w/w) mannitol and 19.9% (w/w) dextran. Five mL Wheaton serum clear glass vials, Lot Number 1192752-02 with rubber stoppers were used to perform the extraction. Vials and syringes were washed in methanol and over dried at 50°C for at least 60 min. Bead filling into vials was performed in the glove box at a relative humidity of 5% at 23°C and sealed with a rubber stopper. All weighing was done in duplicate and the average taken; however, the calculation of percentage of moisture was done individually for each injection, and then

averaged. Water was extracted from the lyophilized beads using methanol. 1.5 mL methanol (~~Riedel-de-Haen~~ RIEDEL-DE HAEN[®] 34741, Lot Number 1085A) was injected into the vial by piecing the rubber stopper with the syringe needle (2.5mL Hamilton gastight syringe with a Mettler Part Number 71483 hypodermic needle Number 12/80). Throughout the extraction period vials were swirled gently to mix the contents at 15 minutes intervals. Injection into the KF titration reaction vessel was performed with a 1 mL Hamilton gastight syringe attached with a Mettler Part Number 71483 hypodermic needle Number 12/80. The syringe was washed with approximately 200 µL of extracted sample prior to injection. A ~~Riedel-de-Haen~~ RIEDEL-DE HAEN[®] #334828, Lot Number 11910 Hydranal Water Standard 1.00 was used to verify the KF performance at the beginning of the run. If the standard gave a result of within 10% of 0.1 % moisture target content then the run was verified as acceptable. The % moisture for each of the five sets of tested lyophilized beads, as well as aggregate statistical information, is provided in Table 5.

[0086] Two types of lyophilized beads were added to the PCR vial in this assay. The first type was a generic bead (GB), which, in addition to the components specified in Example 1, contained a "Hotstart" Platinum PLATINUM[®] DNA polymerase. Hotstart polymerases are precomplexed with specific monoclonal antibodies to render the polymerase inactive. The second bead type was an assay specific bead (ASB), which, in addition to the Example 1 components, also contained primers for *Bacillus Anthracis* and probes. One of each type of bead was placed in the PCR vial. A sample containing lysed *Bacillus Anthracis* bacteria was then added to the PCR vial, along with enough buffer to both dissolve the beads and bring the total reaction volume to 50 µL. In a separate experiment, purified *Bacillus Anthracis* DNA was used. The 50 µL solution was divided into two 25 µL solutions, dispensed into two tubes, and then introduced into a Cepheid Smart-Cycler SMART CYCLER[®] analyzer (Cepheid, Sunnyvale, CA). The solutions were then subjected to an initial heating to 95 °C for 30 seconds. Next, the solutions were subjected to 45 PCR cycles. The PCR cycle parameters were: 95 °C for 1 second and 65 °C for 20 seconds. The initial amount of *Bacillus Anthracis* DNA, the amount of fluorescence observed at the conclusion of 45 cycles (end point), mean end point, number of

cycles required before fluorescence was viewed (cycle threshold), and mean cycle threshold are shown in Table 7.